FORENSIC TOXICOLOGY LABORATORY
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METHOD VALIDATION

PRINCIPLE

The validation process determines whether a given laboratory test method is suited to its intended purpose. Each assay must be evaluated according to its own merits and subject to its intended application. (For example, the criteria for accepting an immunoassay screening test are different from those for a GC/MS quantitation test). Validation parameters include accuracy, precision, linearity, limit of detection, limit of quantitation, system suitability and specificity.

ACCURACY

Accuracy is the agreement between a measured value and the accepted, or true, value. Determination of accuracy requires the comparison of the test method measurements with a known value. This known value can be obtained from a specially prepared (spiked) sample. the results obtained from another test method of known accuracy and precision, or a reference material of known or generally accepted composition.

The optimal evaluation of accuracy is made by analyzing spiked samples. A series of spiked samples, prepared with analytes of known purity, is added to a certified negative sample matrix and analyzed. Alternatively, evaluation of the accuracy of a new method can be made by comparing results to those obtained by a previously accepted method.

PRECISION

Precision is the reproducibility of the test method. Precision requires the agreement between values of two or more measurements that are obtained under identical conditions using the same test method. **Ruggedness** is a component of precision. It is the measure of the test method's ability to withstand minor operating changes in the performance of the assay.

Precision is calculated in terms of the estimated standard deviation for the test method. The determination of estimated standard deviation should be based on as large a number of measurements as possible. Data from all sets of measurements are pooled to determine the overall standard deviation for the measurement process of the assay. Measurements should be generated on multiple aliquots of the same samples analyzed by multiple operators, on multiple days.

An estimate of ruggedness of a test method can be made by evaluating the day to day, analyst to analyst, instrument to instrument variations of the assay. A test method should give reasonably similar results, regardless of the analyst, instrument or date of the assay.

LINEARITY

Linearity is a measure of the correlation between the test method response and the concentration of the analyte. It is obtained either directly or through a well defined mathematical transformation. **Sensitivity** is component of linearity. It can be defined as the ability of the test method to quantitate small changes in analyte concentration, i.e., the ability of the method to differentiate a positive from a negative.

Evaluation of linearity can be better described as the characterization of the test method response curve. A plot of test method response against analyte concentration is often expected to be linear over a specified range of concentrations. Some assays generate non linear response curves.

Data from the analysis of a series of standards are used to characterize the test method response to the concentration curve of the assay. Although it is not necessary to determine the entire range for the test method, the range of concentrations that are used should extend at least beyond the range of concentrations normally used for the assay. A good working range is from 50% of the lowest to150% of the highest normally used concentration.

A linear regression calculation is performed when the assay is expected to be linear. The appropriate curve-fitting calculations are performed when nonlinear response curves are obtained.

LIMIT OF DETECTION (LOD)

The limit of detection is the lowest concentration of analyte present in the sample matrix that is detected, although not necessarily quantitated, under the stated analytical conditions.

The limit of detection is determined by measuring the lowest concentration of analyte that will give an assay response that is significantly different from that of a negative sample (blank). An alternative for chromatographic methods is to require some multiple (usually two or three) of the base line noise.

The limit of detection does not need to be determined for those assays which are used only to measure a known range of concentrations (e.g., vitreous chemistries). It is not correct to state that a sample contains zero analyte, rather, the analyte concentration of the sample is said to be less than the limit of detection of the assay.

The limit of detection can be estimated by extending the calculated assay response curve to the concentration that is equivalent to zero assay response. It is still necessary to prepare a series of samples in this concentration range to determine the actual limit of detection. Multiple measurements are made at concentrations above and below the limit of detection, making sure that there is no sample carry-over.

LIMIT OF QUANTITATION (LOQ)

Limit of quantitation (minimum quantifiable amount) is the lowest concentration of analyte present in the sample matrix that can be quantified with acceptable accuracy and precision. It is possible to estimate the limit of quantitation as the lower limit of the linear range.

SYSTEM SUITABILITY

System suitability is based on the established criteria for any given analysis that must be met in order to accept the data that are generated. In other words, system suitability comprises of criteria established for accepting or rejecting assay results. Acceptance criteria must be consistent with the data evaluated in the validation study.

SPECIFICITY

Specificity is the ability of the test method to measure the analyte without interference from other sample matrix components. **Stability** is a component of specificity. It is the ability of the test method to measure small changes in the analyte concentration without the interference of known degradation products.

Specificity can be demonstrated by showing that components expected to be present on the sample matrix do not interfere with the quantitation of the target analyte.